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13. ABSTRACT (Maximum 200 Words)

Two cohorts of relatively early stage prostate cancer patients, one treated with radiation therapy and the other with surgery have bee identified and have been shown to have similar 5-year disease free survivals after treatment. Abnormal p53 protein levels, indicating mutation, are present in a substantial percentage of relatively early stage prostate cancer patients.

High levels of p53 protein strongly correlates, both under univariate and multivariate analysis, with higher rates of subsequent PSA failure in patients treated with radiation therapy but not in patients treated with radical prostatectomy indicating a predictive power that has significant specificity of radiation.

This study indicates that p53 is a very strong predictor of outcome after radiotherapy but not after surgery in early stage prostate cancer. If pretreatment markers such as p53 that are specific for radiation response can be identified and confirmed in additional clinical trials, their availability could ultimately supplement the medical decision-making process and allow better prospective tailoring of treatment to the biological characteristics of each patient's tumor. For example, a patient predicted to be at high risk for failure specifically after conventional radiotherapy might be better served by surgery or by aggressive dose escalation or perhaps by therapies that targets the identified molecular defect.

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INTRODUCTION

Radiation therapy is a primary treatment modality for clinically localized prostate cancer. Laboratory and clinical evidence, however, suggests substantial heterogeneity in the response of prostate cancer to radiation and it is likely that intrinsic differences in cellular radiosensitivity play a major role. Recent attention has focused on the potential of certain molecular determinants to serve as biological response predictors in human cancer. This study has evaluated the clinical utility of certain candidate markers as specific predictors of prostate cancer response to radiation.

The overall goals of the application have been reached. Accomplishments include: 1) the identification of 80 favorable risk radiation-treated patients with archived biopsy tissue and with at least 5 years of clinical follow-up; 2) the completion of p53 and bcl-2 immunohistochemically detected expression levels in these 80 patients; 3) the completion of p53 and bcl-2 level determinations in 80 clinically similar patients treated instead with a radical prostatectomy; and 4) the completion of the analysis of p53 and bcl-2 versus surgical outcome in these surgery patients with a comparison of the strength of the p53 versus outcome correlation in radiation versus surgery patients. The positive results obtained in this study have been used in a successful NCI RO-1 application that will seek to further validate the findings using pathology samples from a large, multi-institutional clinical trial.

Details of these results are provided below:

RESULTS

Specific aim:

1) To analyze the clinical outcomes in two well-defined groups of patients previously treated with radiotherapy or radical prostatectomy for early stage, favorable-to-intermediate risk prostate cancer. Selected for relatively low pretreatment PSAs and grades, such patients will be likely to have presented with localized disease at the time of diagnosis;

Result: Clinical outcomes (PSA disease free survivals have been determined in 80 patients each who have undergone either radiotherapy or surgery for their early to intermediate stage prostate cancer. As might be expected, disease-free survivals (PSA recurrence free survivals) are similar for the two groups as shown in **figure 1**:

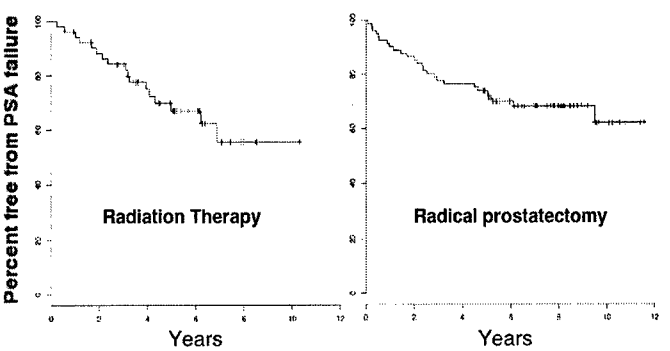


Figure 1. PSA recurrence free survival in cohorts of patients treated with radiation therapy or surgery.

2) To immunohistochemically measure the levels of p53, Bcl-2 and epidermal growth factor receptor (EGFr) in pre-treatment diagnostic biopsy specimens from the same patient cohorts. These markers were selected based upon their potential linkage to radiation response.

Result: Biopsy specimens of patients previously treated with radiation therapy have been analyzed for overexpression of the tumor markers p53, bcl-2 and EGFr. Overexpression of p53 was found to occur in about 38% and 30% of radiation and surgery patients, respectively, whereas bcl-2 abnormal expression occurred in 15.6% and 18.5% of radiation and surgery patients, respectively. EGFr was found to be overexpressed in only about 12% of radiation patients' biopsy samples, rendering it less likely to be useful for predictive purposes **(Fig. 2)**.

The low EGFR overexpression frequency we and others have found dramatically limits any usefulness it could have as a predictive marker. In addition, emerging reports indicatie difficulties in accomplishing meaningful immunohistochemical measurements of EGFR. These two factors led us not carry out any further analyses of EGFR, since a low frequency dramatically limits any usefulness a predictive marker might have.

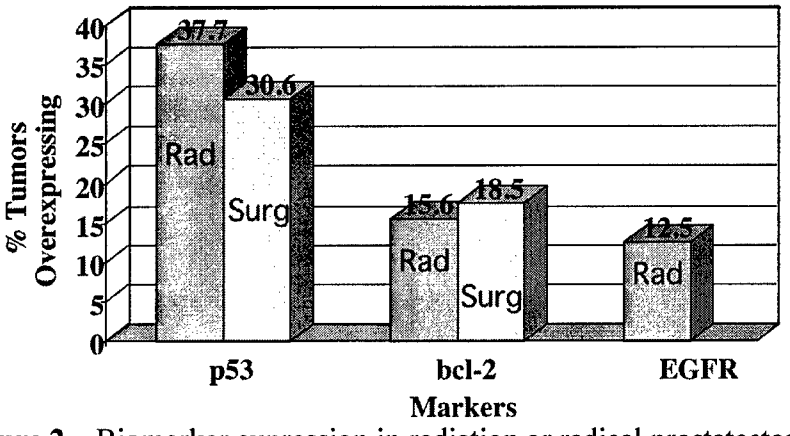
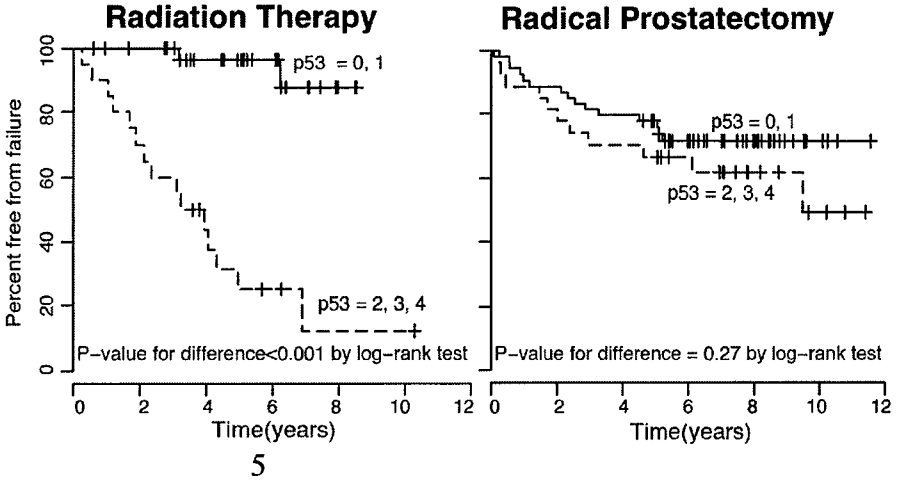


Figure 2. Biomarker expression in radiation or radical prostatectomy patients.

3) To analyze correlations between markers and clinical outcomes in radiation and surgery patients using univariate and multivariate analysis, including conventional prognosticators such as stage, grade and PSA;

Results: It was found that p53 overexpression (> 10% labeling) strongly predicted tumor recurrence in the group of early stage prostate cancer patients treated with radiotherapy, whereas similar studies in the surgical patient cohort of 80 patients indicated not nearly as strong a predictive power for p53 status after surgery (**Fig. 3**). Actuarial PSA recurrence-free survival curves versus p53 status are shown in the figures below for the radiation and the surgery patients. They illustrate a clearly much stronger predictive power of p53 for radiation-treated patients versus surgery patients, a relationship we statistically documented (see Specific aim 4).

Figure 3. p53 scores versus biochemical disease-free survival in the radiation therapy and surgery patient cohorts. (Scoring index: no labeling = 0; 1-10% labeling index = 1; 11–33% = 2; 34 – 66% = 3; 67-100% = 4.)



Multivariate analyses or p53 status versus clinical outcome in radiation and surgery patients were also carried out to include common clinical prognosticators such as Gleason score, initial PSA and stage. As shown in **Tables 1** and **2,** p53 remained a strong predictor of outcome for radiation patients but only a week predictor for surgery patients.

Table 1. Radiation therapy patients: Multi-variate analysis of pre-treatment p53 and clinical parameters vs PSA failure.

Risk factor	Hazard ratio	(95% confidence interval)	p value
Gleason (7 vs. ≤ 6)	3.54	(1.49, 8.38)	0.004
PSA (> 10 vs. ≤ 10)	4.27	(1.82, 10.03)	< 0.001
T stage (T2 vs. T1)	1.39	(0.59, 3.26)	0.450
p53 (2, 3, 4 vs. 0, 1)	1.97	(0.84, 4.62)	0.120

Table 2. Radical prostatectomy patients: Multivariate analysis of pretreatment p53 and clinical parameters vs PSA failure.

Risk factor	Hazard ratio	(95% confidence interval)	p value
Gleason (7 vs. ≤ 6)	3.54	(1.49, 8.38)	0.004
PSA (> 10 vs. ≤ 10)	4.27	(1.82, 10.03)	< 0.001
T stage (T2 vs. T1)	1.39	(0.59, 3.26)	0.450
p53 (2, 3, 4 vs. 0, 1)	1.97	(0.84, 4.62)	0.120

Bcl-2 status was also determined in the radiation and surgery patient cohorts and was not found to be predictive of recurrence in either **(Fig. 4)**,

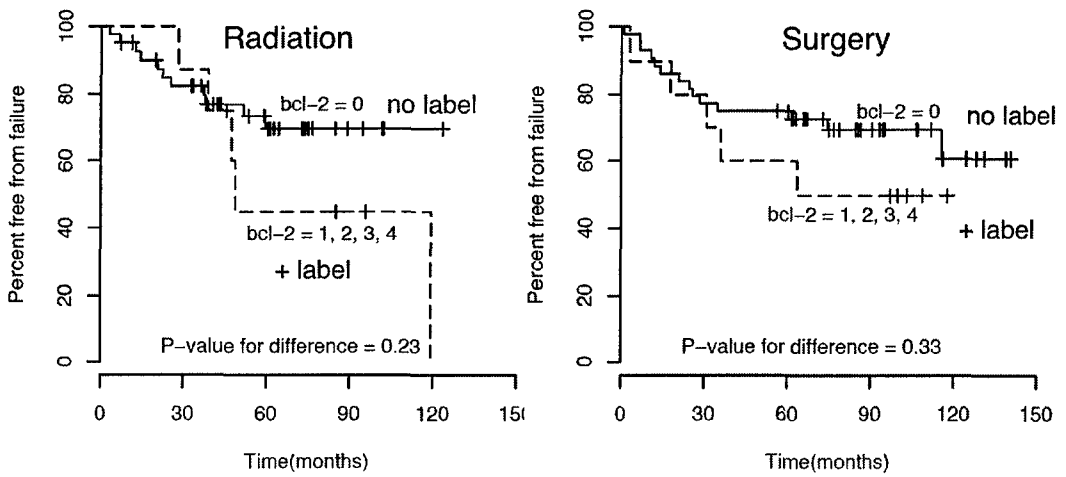


Figure 4. Bcl-2 labeling status versus biochemical disease-free survival in the radiation therapy and surgery patient cohorts. (Scoring index: no labeling = 0; 1-10% labeling index = 1; 11– 33% = 2; 34 – 66% = 3; 67-100% = 4.)

4) To distinguish between predictors of radioresponse and general prognosticators by comparing marker versus outcome data in the radiotherapy versus surgery patient cohorts.

Result: An interaction test was conducted to formalize and solidify the conclusion that the prognostic power of p53 status was greater for irradiated patients than for surgery patients. Merged data sets from the radiation and surgery patients were analyzed with a Cox proportional hazards model that included Gleason score, pretreatment PSA, p53 score and their interactions with treatment modality. It was found

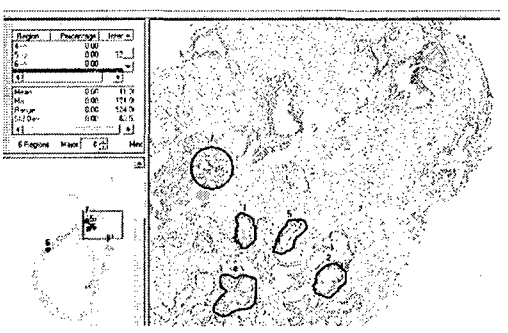
that the changes in hazard ratio as p53 index increases from 0, 1 (≤ 10% labeling) to 2, 3, 4 (>10% labeling) was significantly different between the two treatment groups ($p = 0.007$).

Thus, our studies demonstrate a highly significant correlation between poor outcome and high p53 immunostaining in radiation therapy patients. Furthermore, given the lack of a similarly significant correlation in a control group of surgical patients, it can be concluded that p53 has a strong predictive power for outcomes after radiation therapy that is specific for the radiation therapy modality.

5) Image analysis versus manual counting of immunohistochemistry (IHC) staining.

Potential institution-to-institution variability in manual scoring of IHC staining could hinder the widespread applicability of our findings. Concern over this led us to explore the role of computer assisted image analysis. Recently, we have completed testing of a newer more quantitative IHC analysis method using the Automated Cellular Imaging System® (ACIS) by ChromaVision (San Juan Capistrano, CA). The ACIS is an automated brightfield microscope with digital image processor for analyzing fixed and stained cellular specimens on glass microscope slides. The ACIS consists of a robotic microscope module, a computer and peripheral accessories. For analysis, stained sample slides are placed in plastic carriers, which are automatically transported across the microscope stage for viewing. Tissue array slides can be scanned as well. The system can detect, count, and classify cells based on size, color, intensity of staining and shape. Since the entire slide is scanned and the entire image stored, the pathologist can later review the computer identified "hot spot" areas of IHC staining, define these further and instantly obtain staining parameter information. The ACIS system may substantially improve the reproducibility in our quantitative IHC analysis.

Correlation between manual counting and ACIS. We have carried out a preliminary analysis of ACIS to manual IHC labeling indices on p53 stained slides and the results are shown below:



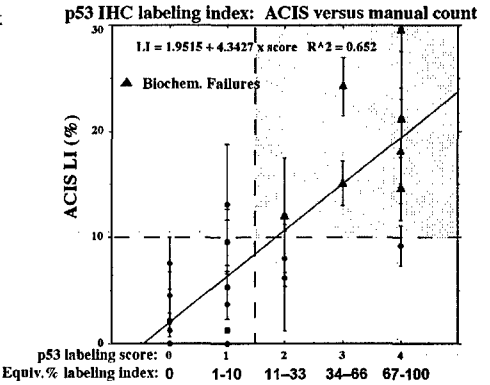


Figure 5. (Left) ACIS workstation analysis desktop showing a low power scan on the left, from which an area of interest is chosen for further analysis of p53 labeling on the right. Outlined areas are then thresholded and scored in automated fashion for labeling.

Figure 6. (Right) ACIS-determined p53 IHC labeling indices +/- standard deviations versus hand count indices. Biochemical failures were reproducibly segregated by a labeling index threshold of 10% with each method (shaded area).

ACIS-determined labeling indices were compared with the hand-counted scoring index used in that study. Figure **5** is an example of the ACIS workstation analysis screen in which a section of the low power scan on the left is chosen for further analysis. Tumor containing regions are outlined, thresholds are set and automated counting provides measurements of labeling index with standard deviation, as

well as stain density if relevant to the study. Correlation between manual counts and ACIS was excellent (**Figure 6**) and all 7 biochemical failures in this limited data set occurred for p53 labeling indices >10%, whether scored by ACIS or manual counting (shaded area on right). We intent to further develop and implement this technology in future studies.

KEY RESEARCH ACCOMPLISHMENTS

- Two cohorts of relatively early stage prostate cancer patients, one treated with radiation therapy and the other with surgery, have been identified and have been shown to have similar 5-year disease free survivals after treatment.
- Abnormal p53 protein levels, indicating mutation, are present in a substantial percentage of relatively early stage prostate cancer patients.
- High levels of p53 protein strongly correlates, both under univariate and multivariate analysis with higher rates of subsequent PSA failure in patients treated with radiation therapy but not in patients treated with radical prostatectomy, indicating a predictive power that has significant specificity for radiation. This finding could lead to better prospective tailoring of therapy to each patient's gumor characteristics in the future.
- Overexpression of bcl-2, an apoptotic pathway marker, has only weak predictive power for radiation control of prostate cancer and loses its significance in a multivariate analysis that includes p53 status.
- Computer-assisted image analysis was preliminarily explored and found to be promising as an alternative to manual counting of immunohistochemically stained slides.

REPORTABLE OUTCOMES:

- Ritter MA, Gilchrist K, Voytovich M, Verhovan B: p53 as a predictor of radiation response in prostate cancer - early stage disease. Int J Radiat Oncol Biol Phys 53:574-80, 2002.
- "Radiation Therapy Outcomes and p53 Status for Favorable-to-Intermediate Risk Prostate Cancer" 2nd International Meeting on Cancer Diagnostics, NCI-EORTC, June 26-29, 2002.
- Integrating biomarkers in the prognostic algorithm for early stage prostate cancer, American Radium Society, May, 2004
- Predictive versus prognostic biomarkers in localized prostate cancer treated with radiotherapy. In preparation.

CONCLUSIONS:

The results of this study indicate that p53 is a very strong predictor of outcome after radiotherapy but not after surgery in early stage prostate cancer. If pretreatment markers such as p53 that are specific for radiation response can be identified and validated in additional clinical trials, their availability could ultimately supplement the medical decision-making process and allow a better prospective tailoring of treatment to the biological characteristics of each patient's tumor. For example, a patient predicted to be at high risk for failure after conventional radiotherapy might be better served by surgery or by aggressive dose escalation or perhaps by therapies that targets the identified molecular defect.

The results obtained in this DOD-funded study have served to provide a significant portion of the preliminary studies in a recently awarded and soon to begin NCI RO-1 application that will explore the predictive power of p53 and other markers in a much larger group of patients treated in a national cooperative trial.

REFERENCES: NONE

APPENDIX

Ritter MA, Gilchrist K, Voytovich M, Verhovan B: p53 as a predictor of radiation response in prostate cancer – early stage disease. Int J Radiat Oncol Biol Phys 53:574-80, 2002.

Attached as a PDF file.

Int. J. Radiation Oncology Biol. Phys., Vol. 53, No. 3, pp. 574–580, 2002

0360-3016/02/$–see front matter

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PII S0360-3016(02)02781-5

CLINICAL INVESTIGATION **Prostate**

THE ROLE OF p53 IN RADIATION THERAPY OUTCOMES FOR FAVORABLE-TO-INTERMEDIATE–RISK PROSTATE CANCER

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Purpose: Some prostate cancers may have molecular alterations that render them less responsive to radiation therapy; identification of these alterations before treatment might allow improved treatment optimization. This study investigated whether p53, a potential molecular determinant, could predict long-term radiation therapy outcome in a restricted group of relatively favorable-risk prostate cancer patients treated uniformly with irradiation alone.

Methods and Materials: This study included 53 patients previously treated with radiotherapy for favorable-to-intermediate–risk prostate cancer. These patients were selected for relatively low pretreatment PSAs (≤21 ng/mL) and Gleason scores (≤7) to decrease the likelihood of nonlocalized disease, because disease localization was necessary to examine the efficacy of localized radiation therapy. The status of p53 was immunohistochemically assessed in paraffin-embedded pretreatment biopsy specimens, along with appropriate controls. This marker was selected based upon a usable mutation prevalence in early-stage prostate cancer and its potential linkage with radiation response via cell cycle, DNA repair, and cell death pathways. Correlation between p53 mutation and clinical outcome was analyzed in univariate and multivariate fashion and included conventional prognosticators, such as stage, grade, and PSA. Freedom from biochemical failure was determined using American Society for Therapeutic Radiology and Oncology criteria. Limitations of prior studies were potentially avoided by requiring adequate posttreatment follow-up (median follow-up in nonfailing patients of 5.1 years), as well as pretreatment PSA and Gleason scores that suggested localized disease, and uniformity of treatment.

Results: The total group of 53 favorable-to-intermediate–risk patients demonstrated an actuarial biochemical failure rate of 35% at 5 years. Forty percent of all specimens had a greater than 10% labeling index for p53 mutation, and actuarial biochemical control was found to strongly and independently correlate with p53 status. Patients with higher p53 labeling indices demonstrated significantly higher PSA failure rates ($p < 0.001$). In contrast, p53 status did not correlate with pretreatment PSA, grade, or tumor stage. Similarly, pretreatment PSA (log–rank 0.22), Gleason score (log–rank 0.93), and T stage (log–rank 0.15) were not prognostic for outcome in this group of patients selected for their relatively favorable clinical characteristics.

Conclusions: (*1*) p53 status in pretreatment biopsies strongly predicted for long-term biochemical control after radiation therapy in favorable-to-intermediate–risk prostate cancer patients. (*2*) If validated in other independent clinical data sets, p53 status should be considered as a stratification factor in future clinical trials and could be useful in guiding treatment. Abnormal p53 status might favor surgical management, aggressive dose escalation, or p53-targeted therapy. © 2002 Elsevier Science Inc.

Prostatic neoplasms, Radiotherapy, p53, Prognostic factors.

INTRODUCTION

Prostate cancer is the most common nonskin cancer in American men, resulting in more than 30,000 deaths annually in the United States. Despite favorable toxicity profiles and outcomes that may be comparable to those obtained with radical prostatectomy, clinical outcomes after radiation therapy still suggest that local tumor recurrence remains a numerically and clinically important mode of treatment failure (1). Although radiation combined with anti-androgen therapy (2, 3) and conformal dose escalation (4–9) can improve clinical outcome, it would be clinically useful to identify and make prospective use of markers of radiation response.

Pretreatment prostate-specific antigen (PSA), tumor grade, and stage predict for clinical outcome, irrespective of treatment type (10); however, predictors that are specific for radiation response have not been available. Some prostate cancers may have molecular alterations that render them poorly responsive to radiation therapy and that contribute to many of the treatment failures observed after radiation

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therapy. This study investigated whether one such potential molecular determinant, p53, could predict long-term radiation therapy outcome in a selected group of favorable-to-intermediate–risk prostate cancer patients previously treated in uniform fashion with small-field irradiation alone. The choice of p53 was predicated upon its potentially central role in radiation response (11), the existence of some limited clinical correlative studies suggesting that abnormal p53 function predicts for poor radiation therapy outcomes in prostate cancer (12–16), and, lastly, the significant prevalence of p53 mutations in early-stage prostate cancer (12, 13, 16). Furthermore, in the great majority of prostate cancer cases, p53 mutations result in an overaccumulation of functionally inactive p53 protein (17), an accumulation that can be detected using a clinically implementable immunohistochemical approach (18).

This study attempted to minimize potential limitations of several previous studies by requiring adequate posttreatment follow-up (median of 5.1 years in nonfailing patients), uniformity of treatment (no hormonal therapy), and lower pretreatment PSA and Gleason scores, consistent with localized disease. The efficacy of radiation therapy can, of course, be adequately tested only in patients with a high initial likelihood of localized disease. This study's inclusion criteria are clinically relevant in that they mirror the clinical characteristics with which most contemporary prostate cancer patients present.

METHODS AND MATERIALS

Patient selection

A cohort of 67 patients uniformly treated for localized prostate cancer between 1988 and was identified with pretreatment PSAs ≤21 ng/mL, Gleason scores ≤7, and pathology specimens available at our institution. Of these specimens, 14 had insufficient tumor to allow immunohistochemical analysis. The clinical characteristics of the remaining 53 patients (41 needle biopsies and 12 transurethral resections of the prostate) are summarized in Table 1; these patients form the basis for this study. These patients were treated only with small-field radiation therapy, to minimum prostate doses of between 68 and 72 Gy. Because pretreatment PSA and Gleason scores are strong predictors of nonlocalized disease, the selection of patients with relatively favorable values was expected to increase the likelihood of only localized disease at presentation. This condition was necessary to test the efficacy of radiation and the power of certain markers to predict that efficacy. The year 1988 was the earliest for which pretreatment PSAs were routinely available. The selection of early 1995 as a cutoff for eligibility allows for adequate minimum follow-up of clinical outcome. The median follow-up in nonfailing patients was 5.1 years. Clinical outcome was assessed as biochemical (PSA) disease-free survival. PSA failures were defined according to American Society for Therapeutic Radiology and Oncology consensus recommendations (19).

Table 1. Patient characteristics

Parameters	No. of patients (%)	Median	Total range
PSA (ng/mL)		9.0	1.3–21
Range			
3–4	10		
>4–10	21		
>10–15	14		
>15–21	8		
Gleason score		5.5	3–7
Range			
≤4	15		
5–6	29		
7	9		
T stage			
T1	22 (41)		
T2	30 (57)		
T3	1 (2)		

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded blocks from the original diagnostic biopsies. Portions of blocks were sectioned at 5 μm and mounted on slides. One slide from a central section was H&E stained, and the adjacent slides were used for immunohistochemical staining for p53 (clone BP53-12 NeoMarkers, Inc.). Heat-induced epitope retrieval was accomplished using an electric pressure cooker (Decloaking Chamber, BioCare Medical), and slides were stained on an automated immunohistochemistry stainer (Ventana Medical Systems, Inc.). Slides were then lightly counterstained with hematoxylin and scored for the p53 labeling index.

DU-145, PC-3, and PC-3 xenograft tumors were included in staining runs to serve as graded positive and negative controls. The scoring system used is shown in Table 2: By testing, we found that this set of controls, combined with this scoring system, provides scoring consistency over multiple, independent determinations (data not shown).

Statistical analyses

Data were evaluated for disease-specific survival using the Kaplan–Meier product limit method, the log–rank test, and multivariate analysis in a Cox proportional hazards model for markers and other recognized clinical and pathologic predictors of outcome. Deaths due to intercurrent disease were considered losses to follow-up. Predictors were modeled as binary (stage, Gleason score, and PSA) or continuous (p53 score) variables.

Table 2. Immunohistochemistry scoring system for p53

Scoring index	% labeled
0	0
1	1–10
2	11–33
3	34–66
4	67–100

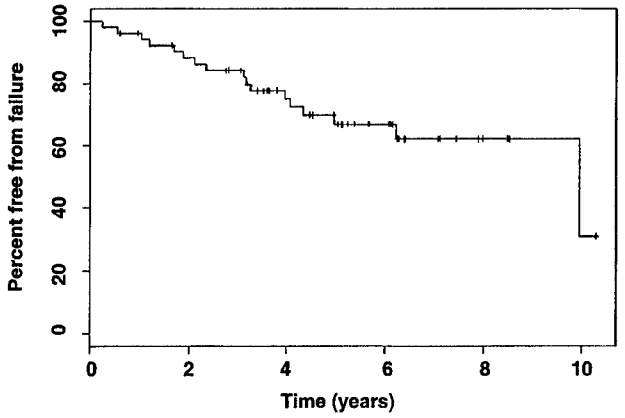

Fig. 1. PSA recurrence-free survival in the total group of 53 patients.

RESULTS

Diagnostic biopsy tissue blocks that were available at our institution and that were from patients meeting the PSA, grade, and treatment date and type eligibility requirements for the study were identified for a group of 53 patients. The clinical outcome of this entire group is shown in Fig. 1, which illustrates an actuarial 35% PSA failure rate at 5 years using American Society for Therapeutic Radiology and Oncology's criteria for PSA failure (19). A total of 17 patients experienced a PSA failure.

p53 analysis

p53 indices were immunohistochemically measured in these 53 previously treated patients, and correlations with standard prognosticators (grade, stage, and PSA) and clinical outcome were analyzed. Twenty patients (37%) had a greater than 10% labeling index (score ≥2), as indicated in Fig. 2.

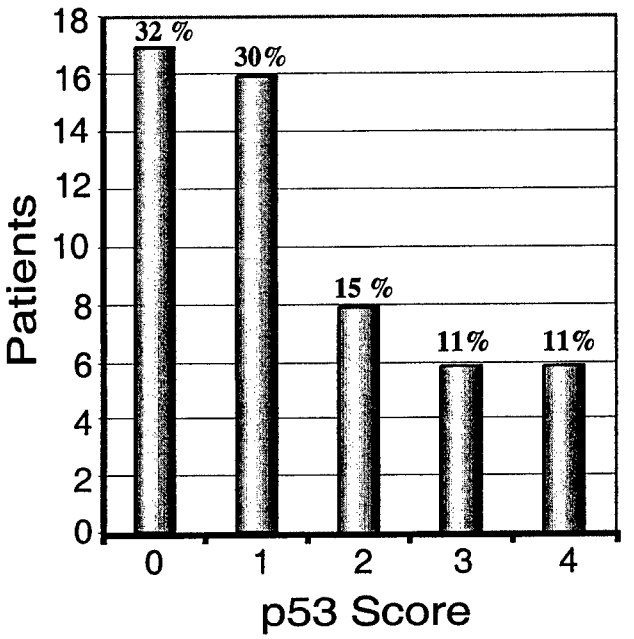

Fig. 2. Distribution of p53 immunohistochemical labeling indices in 53 patients. Score 0 = 0%, score 1 = 1%–10%, score 2 = 11%–33%, score 3 = 34%–66%, and score 4 = 67%–100%.

Low correlation was seen between p53 and pretreatment PSA, grade, or tumor stage, which is the expected result of restricting the PSA, grade, and stage entry criteria for this study. However, clinical outcome measured by PSA control was found to strongly correlate with p53 status. Patients whose tumors demonstrated a greater than 10% p53 labeling index (a scoring index ≥2) demonstrated a 5-year actuarial

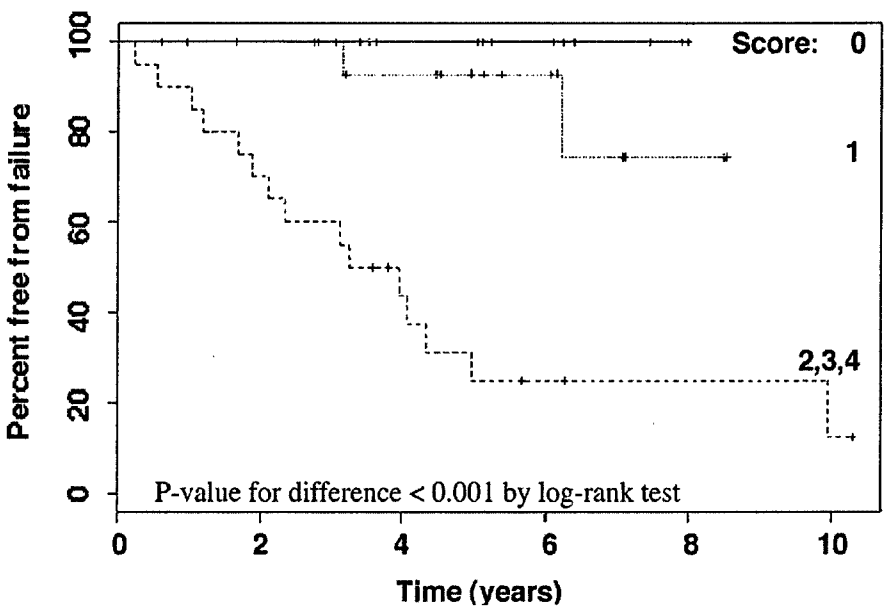

Fig. 3. Biochemical disease-free survival vs. p53 labeling score in 53 patients.

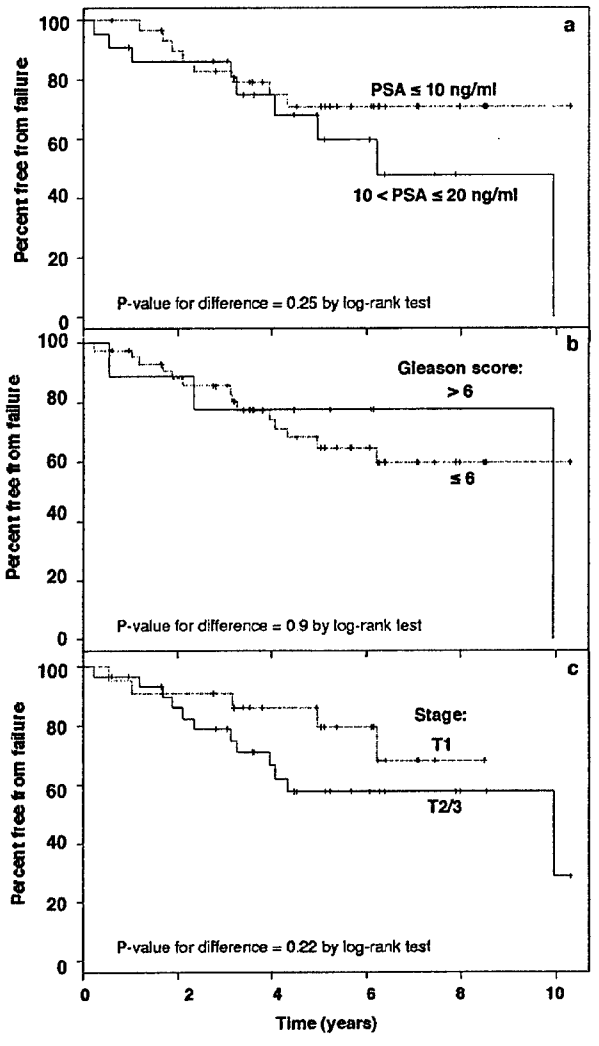

Fig. 4. Biochemical disease-free survival vs. (a) pretreatment PSA, (b) Gleason score, or (c) T stage.

biochemical failure rate of 76% (Fig. 3). In contrast, those patients with p53 labeling indices of 10% or less (score ≤1) experienced a biochemical failure rate of only 5% (log–rank, $p < 0.001$). There was a nonsignificant trend toward a somewhat poorer outcome for score 1 vs. score 0 patients with longer follow-up. Of particular note is that no patient with an undetectable p53 labeling index experienced a failure within the follow-up time frame of this study. In fact, a simpler scoring system consisting of zero vs. nonzero p53 labeling (score 0 vs. score ≥1) predicted for actuarial estimated 5-year biochemical disease-free survivals of 100% and 54%, respectively ($p = 0.004$).

Pretreatment PSA, Gleason score, or T stage were not prognostic for outcome in this group of patients, having narrowly defined pretreatment characteristics (Fig. 4).

A multivariate analysis was performed that included p53 status, grade, stage, and PSA versus biochemical control. The outcomes of this analysis, including relative risks, are shown in Table 3. It is clear that p53 status is the only variable that had independent prognostic significance in this group of patients. Thus, our results demonstrate a highly significant correlation between poor outcome and high p53 immunostaining.

DISCUSSION

Recent attention has focused on the potential for certain molecular determinants to serve as biologic response predictors in human cancer. Such studies have indicated that the status of biologic markers such as p53, bax, bcl-2, or epidermal growth factor receptor can influence response to radiation in cancers of the breast (20–23), head and neck (24–28), and lung (29, 30). The status of p53 has also been found to alter the *in vitro* (31) and *in vivo* (32) radiation response of prostate cancer cells.

There is also preliminary clinical data for prostate cancer indicating links between radiation response and certain molecular markers. Clinical correlative studies in prostate cancer patients treated with radiation have suggested correlations between outcome and the status of p53, bcl-2, and bax in their tumors (12–16, 33, 34). Markers were determined either in pretreatment biopsies or in material obtained at the time of local tumor recurrence. With one exception (34), immunohistochemically detected abnormal levels of these markers correlated with increased local recurrence. Elevated immunohistochemically detected levels correlated with increased recurrence, except for bax, for which the inverse applied. A summary of these studies is provided in Table 4.

The tumor suppressor gene p53 has been the most extensively studied of these markers in the radiotherapy of prostate cancer, but no studies to date have conclusively linked p53 to radiation response in a clinically useful fashion. Existing studies have provided intriguing clues. However, many studies cited in Table 4 are weakened by low enrollment or the inclusion of patients with

Table 3. Multivariate analysis of clinical and p53 score parameters vs. biochemical control

Risk factor	Hazard ratio	(95% confidence interval)	*p* value
p53 (per unit IHC score increase)	2.3	(1.6, 3.5)	<0.0001
T2/3 (vs. T1)	1.1	(0.3, 3.2)	0.9
Gleason (7 vs. ≤6)	0.7	(0.16, 2.8)	0.6
PSA (>10 vs. ≤10)	2.0	(0.7, 5.5)	0.17

Abbreviation: IHC = immunohistochemistry.

Table 4. Previous studies of p53 and radiotherapy outcomes in prostate cancer

Marker	No. of patients	Predicts failure?	When assessed?	References
p53 Bcl-2	54	+ +	Pretreatment	(12)
p53 GST-pi	55	+ +	At recurrence	(13)
p53	13	+	At recurrence	(14)
Bcl-2 p53	43 pre-RT; 53 post-RT	+ +/−	Both	(33)
Bcl-2	42	−	Pretreatment	(34)
Bcl-2/bax	41	+	Pretreatment	(15)
p53	129	+	Pretreatment	(16)

Abbreviation: RT = radiotherapy.

a broad range of pretreatment prognoses (including very high PSAs, high tumor grades, and even hormonally resistant disease, in some cases) or patients treated with hormonal therapy in addition to radiation. Thus, although the studies suggest predictive, correlative relationships, none conclusively demonstrate predictive capability in the treatment of early-stage prostate cancer with radiation therapy alone.

We attempted to address these issues in our investigation by including only patients with narrowly defined pretreatment characteristics that increased the likelihood of localized disease at the time of treatment. Only patients who had received radiation therapy alone were included. We chose to focus on p53 because of the previous studies suggesting a predictive role for p53 in prostate radiation response and, also importantly, because p53 mutation frequencies are reported to occur in early-stage prostate cancer at an estimated frequency of 20% to 35% (12, 13, 16), sufficiently high enough to make any predictor of outcome clinically useful. Clearly, an infrequently abnormal marker, even if highly correlated with radiosensitivity, would be of little clinical utility.

In addition, there is a strong biologic basis for considering p53 status as a radiation predictor. It has been extensively described as a central mediator of cellular response to DNA-damaging agents, with involvement in induction of the apoptotic response, DNA repair, and cell cycle delay (11). DNA damage induces an increase in p53 protein levels, resulting in the potential activation of numerous molecular pathways. These include transcriptional activation of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$, which potentiates cell cycle arrest (35), as well as activation of GADD45 and its DNA repair–related activities (36). p53 can also induce transcriptional activation of bax (37), thereby promoting apoptosis. Numerous *in vitro* experiments that have manipulated cellular p53 status have found increased resistance to the cytotoxic effects of radiation or chemotherapy when p53 function is disabled (38, 39). Additionally, alterations in p53 function have been shown to reduce cell doubling times (40, 41), a change that might increase tumor clonogen repopulation during multiple-fraction radiation therapy. These findings suggest that dysfunctional p53 will reduce tumor control by radiation. However, because response to ionizing radiation likely involves a number of p53-mediated events that themselves require the integration of both intracellular and extracellular signals, the precise impact of p53 status upon radiosensitivity could vary with, and should be determined in, each type of tumor.

It has been found in the great majority of cases that p53 mutations in prostate cancer result in an overaccumulation of functionally inactive p53 protein (17), which can be detected using an immunohistochemical approach (18). Whereas genomic alterations will certainly be relevant to differential responses to agents such as radiation, the investigation of downstream differences at the protein level takes into account intervening posttranslational processes. Immunohistochemistry remains one of the more clinically practical methods of doing so. Although nonquantitativeness and poor reproducibility can challenge the reliability of this approach, a careful standardization of technique and the use of appropriate, concurrent positive and negative controls can produce a more stable and reliable analysis. Furthermore, as described earlier, we found that even a simplified binary scoring system (zero vs. nonzero p53 labeling) could predict markedly different clinical outcomes.

By design, this study used diagnostic needle biopsies or transurethral resection specimens that can be subject to sampling errors, but such uncertainties apply to virtually all prostate cancer patients treated definitively with radiation therapy. Additionally, although biochemical failure was used as a surrogate for local failure, the entry restrictions in this study were likely to substantially increase the probability that PSA failure reflected local failure.

In conclusion, it was found that p53 status in pretreatment diagnostic specimens strongly predicted for long-term biochemical control after conventional-dose radiation therapy in favorable-to-intermediate–risk prostate cancer patients. The clinical characteristics of patients included in this study are quite similar to those of typical

patients contemporarily diagnosed with prostate cancer. Should these results be further validated in independent data sets, p53 status could be considered as a stratification factor in future clinical trials of low-to-intermediate–risk prostate cancer and could eventually be useful in guiding therapy. An abnormal p53 status might suggest the consideration of surgical management, aggressive radiation dose escalation, or p53-targeted therapy.

REFERENCES

1. Kuban DA, el-Mahdi AM, Schellhammer P. The significance of post-irradiation prostate biopsy with long-term follow-up. *Int J Radiat Oncol Biol Phys* 1992;24(3):409–414.
2. Pilepich MV, Krall JM, al-Sarraf M, *et al.* Androgen deprivation with radiation therapy compared with radiation therapy alone for locally advanced prostatic carcinoma: A randomized comparative trial of the Radiation Therapy Oncology Group. *Urology* 1995;45(4):616–623.
3. Bolla M, Gonzalez D, Warde P, *et al.* Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. *N Engl J Med* 1997;337(5):295–300.
4. Hanks GE, Lee WR, Hanlon AL, *et al.* Conformal technique dose escalation for prostate cancer: Biochemical evidence of improved cancer control with higher doses in patients with pretreatment prostate-specific antigen > or = 10 NG/ML. *Int J Radiat Oncol Biol Phys* 1996;35(5):861–868.
5. Pollack A, Zagars GK. External beam radiotherapy dose response of prostate cancer. *Int J Radiat Oncol Biol Phys* 1997;39(5):1011–1018.
6. Zelefsky MJ, Leibel SA, Gaudin PB, *et al.* Dose escalation with three-dimensional conformal radiation therapy affects the outcome in prostate cancer [see comments]. *Int J Radiat Oncol Biol Phys* 1998;41(3):491–500.
7. Roach M, 3rd, Meehan S, Kroll S, *et al.* Radiotherapy for high grade clinically localized adenocarcinoma of the prostate [see comments]. *J Urol* 1996;156(5):1719–1723.
8. Vicini FA, Kestin LL, Martinez AA. The importance of adequate follow-up in defining treatment success after external beam irradiation for prostate cancer. *Int J Radiat Oncol Biol Phys* 1999;45(3):553–561.
9. Pollack A, Zagars GK, Smith IG, Antolak JA, Rosen II. Preliminary results of a randomized dose-escalation study comparing 70 Gy to 78 Gy for the treatment of prostate cancer. *Int J Radiat Oncol Biol Phys* 1999;45(Suppl. 3):146a–147a.
10. Partin AW, Kattan MW, Subong EN, *et al.* Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. *JAMA* 1997;277(18):1445–1451.
11. Bristow RG, Benchimol S, Hill RP. The p53 gene as a modifier of intrinsic radiosensitivity: Implications for radiotherapy. *Radiother Oncol* 1996;40(3):197–223.
12. Scherr DS, Vaughan ED, Jr, Wei J, *et al.* BCL-2 and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. *J Urol* 1999;162(1):12–16.
13. Cheng L, Sebo TJ, Cheville JC, *et al.* p53 protein overexpression is associated with increased cell proliferation in patients with locally recurrent prostate carcinoma after radiation therapy. *Cancer* 1999;85(6):1293–1299.
14. Grossfeld GD, Olumi AF, Connolly JA, *et al.* Locally recurrent prostate tumors following either radiation therapy or radical prostatectomy have changes in Ki-67 labeling index, p53 and bcl-2 immunoreactivity. *J Urol* 1998;159(5):1437–1443.
15. Mackey TJ, Borkowski A, Amin P, Jacobs SC, Kyprianou N. bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. *Urology* 1998;52(6):1085–1090.
16. Grignon DJ, Caplan R, Sarkar FH, *et al.* p53 status and prognosis of locally advanced prostatic adenocarcinoma: A study based on RTOG 8610. *J Natl Cancer Inst* 1997;89(2):158–165.
17. Rovinski B, Benchimol S. Immortalization of rat embryo fibroblasts by the cellular p53 oncogene. *Oncogene* 1988;2(5):445–452.
18. Wertz IE, Deitch AD, Gumerlock PH, Gandour-Edwards R, Chi SG, de Vere White RW. Correlation of genetic and immunodetection of TP53 mutations in malignant and benign prostate tissues. *Hum Pathol* 1996;27(6):573–580.
19. ASTRO Panel Consensus statement: Guidelines for PSA following radiation therapy. *Int J Radiat Oncol Biol Phys* 1997;37:1035–1041.
20. Chiarugi V, Magnelli L, Cinelli M. Role of p53 mutations in the radiosensitivity status of tumor cells. *Tumori* 1998;84(5):517–520.
21. Meyn MS. Ataxia-telangiectasia and cellular responses to DNA damage. *Cancer Res* 1995;55(24):5991–6001.
22. Meyn RE, Stephens LC, Mason KA, Medina D. Radiation-induced apoptosis in normal and pre-neoplastic mammary glands in vivo: Significance of gland differentiation and p53 status. *Int J Cancer* 1996;65(4):466–472.
23. Sakakura C, Sweeney EA, Shirahama T, *et al.* Overexpression of bax sensitizes human breast cancer MCF-7 cells to radiation-induced apoptosis. *Int J Cancer* 1996;67(1):101–105.
24. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res* 1999;59(8):1935–1940.
25. Nogueira CP, Dolan RW, Gooey J, *et al.* Inactivation of p53 and amplification of cyclin D1 correlate with clinical outcome in head and neck cancer. *Laryngoscope* 1998;108(3):345–350.
26. Raybaud-Diogene H, Fortin A, Morency R, Roy J, Monteil RA, Tetu B. Markers of radioresistance in squamous cell carcinomas of the head and neck: A clinicopathologic and immunohistochemical study. *J Clin Oncol* 1997;15(3):1030–1038.
27. Servomaa K, Kiuru A, Grenman R, Pekkola-Heino K, Pulkkinen JO, Rytomaa T. p53 mutations associated with increased sensitivity to ionizing radiation in human head and neck cancer cell lines. *Cell Prolif* 1996;29(5):219–230.
28. Sheridan MT, O'Dwyer T, Seymour CB, Mothersill CE. Potential indicators of radiosensitivity in squamous cell carcinoma of the head and neck. *Radiat Oncol Investig* 1997;5(4):180–186.
29. Biard DS, Martin M, Rhun YL, *et al.* Concomitant p53 gene mutation and increased radiosensitivity in rat lung embryo epithelial cells during neoplastic development. *Cancer Res* 1994;54(13):3361–3364.
30. Sirzen F, Zhivotovsky B, Nilsson A, Bergh J, Lewensohn R. Spontaneous and radiation-induced apoptosis in lung carcinoma cells with different intrinsic radiosensitivities. *Anticancer Res* 1998;18(2A):695–699.
31. Colletier PJ, Ashoori F, Cowen D, *et al.* Adenoviral-mediated p53 transgene expression sensitizes both wild-type and null p53 prostate cancer cells in vitro to radiation. *Int J Radiat Oncol Biol Phys* 2000;48(5):1507–1512.

32. Cowen D, Salem N, Ashoori F, *et al.* Prostate cancer radiosensitization in vivo with adenovirus-mediated p53 gene therapy. *Clin Cancer Res* 2000;6(11):4402–4408.
33. Huang A, Gandour-Edwards R, Rosenthal SA, Siders DB, Deitch AD, White RW. p53 and bcl-2 immunohistochemical alterations in prostate cancer treated with radiation therapy. *Urology* 1998;51(2):346–351.
34. Bylund A, Stattin P, Widmark A, Bergh A. Predictive value of bcl-2 immunoreactivity in prostate cancer patients treated with radiotherapy. *Radiother Oncol* 1998;49(2):143–148.
35. Dulic V, Kaufmann WK, Wilson SJ, *et al.* p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell* 1994;76(6):1013–1023.
36. Smith ML, Chen IT, Zhan Q, *et al.* Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* 1994;266(5189):1376–1380.
37. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995; 80(2):293–299.
38. Siles E, Villalobos M, Valenzuela MT, *et al.* Relationship between p53 status and radiosensitivity in human tumour cell lines. *Br J Cancer* 1996;73(5):581–588.
39. Li CY, Nagasawa H, Dahlberg WK, Little JB. Diminished capacity for p53 in mediating a radiation-induced G1 arrest in established human tumor cell lines. *Oncogene* 1995;11(9): 1885–1892.
40. Langendijk JA, Thunnissen FB, Lamers RJ, de Jong JM, ten Velde GP, Wouters EF. The prognostic significance of accumulation of p53 protein in stage III non-small cell lung cancer treated by radiotherapy. *Radiother Oncol* 1995;36(3):218–224.
41. Bourhis J, Bosq J, Wilson GD, *et al.* Correlation between p53 gene expression and tumor-cell proliferation in oropharyngeal cancer. *Int J Cancer* 1994;57(4):458–462.